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(FILE 'HOME' ENTERED AT 17:04:23 ON 15 MAR 2003)

6 S L4 AND AMYLOSE (W) FREE

FILE 'CAPLUS, USPATFULL, AGRICOLA, APOLLIT, BABS, CBNB, CEN, CIN, EMA, IFIPAT, JICST-EPLUS, PASCAL, PLASNEWS, PROMT, RAPRA, SCISEARCH, TEXTILETECH, USPAT2, WPIDS, WTEXTILES, ALUMINIUM, ANABSTR, AQUIRE, BIOCOMMERCE, BIOTECHNO, CABA, CAOLD, CEABA-VTB, ...' ENTERED AT 17:04:58 ON 15 MAR 2003

L1	16705	S	AMYLOPECTIN							
L2	341	S	L1	AND	CASSAVA					
L3	62	S	L2	AND	STARCH	CONTENT				
L4	35	S	L3	AND	TUBER					

L5

(FILE 'HOME' ENTERED AT 17:04:23 ON 15 MAR 2003)

FILE 'CAPLUS, USPATFULL, AGRICOLA, APOLLIT, BABS, CBNB, CEN, CIN, EMA, IFIPAT, JICST-EPLUS, PASCAL, PLASNEWS, PROMT, RAPRA, SCISEARCH, TEXTILETECH, USPAT2, WPIDS, WTEXTILES, ALUMINIUM, ANABSTR, AQUIRE, BIOCOMMERCE, BIOTECHNO, CABA, CAOLD, CEABA-VTB, ...' ENTERED AT 17:04:58 ON 15 MAR 2003

L1	7	6'	7 N	5	S	ΔΜ	V.	r.0	DH	CT	M

L2

L3

341 S L1 AND CASSAVA

62 S L2 AND STARCH CONTENT

L4 35 S L3 AND TUBER

L5 6 S L4 AND AMYLOSE (W) FREE

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```
L5
     ANSWER 1 OF 6 USPATFULL
AN
       2002:262508 USPATFULL
       Nucleic acid molecules from wheat, transgenic plant cells and plants and
TI
       the thereof for the production of modified starch
       Abel, Gernot, Copenhagen, DENMARK
IN
       Lorz, Horst, Hamburg, GERMANY, FEDERAL REPUBLIC OF
       Lutticke, Stephanie, Hamburg, GERMANY, FEDERAL REPUBLIC OF
       Schmidt, Ralf-Christian, Potsdam, GERMANY, FEDERAL REPUBLIC OF
       Aventis CropScience GmbH, Frankfurt am Main, GERMANY, FEDERAL REPUBLIC
PA
       OF (non-U.S. corporation)
PΙ
       US 6462256
                          B1
                               20021008
ΑI
       US 2000-590101
                               20000608 (9)
       DE 1999-19926771
                           19990611
PRAI
DT
       Utility
FS
       GRANTED
LN.CNT 1973
INCL
       INCLM: 800/284.000
       INCLS: 800/298.000; 435/468.000; 435/101.000; 435/419.000; 435/430.000;
              435/252.300; 435/320.100; 435/194.000; 536/023.200; 536/236.000
NCL
              800/284.000
       NCLS:
              435/101.000; 435/194.000; 435/252.300; 435/320.100; 435/419.000;
              435/430.000; 435/468.000; 536/023.200; 536/023.600; 800/298.000
IC
       [7]
       ICM: A01H005-00
       ICS: A01H005-10; C12P019-04; C12N001-21; C12N005-04; C12N009-12;
       C12N015-29; C12N015-54; C12N015-82
EXF
       536/23.2; 536/23.6; 435/468; 435/419; 435/320.101; 435/430; 435/252.3;
       435/194; 800/286; 800/284; 800/278; 800/298
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM
               of branching of the glucose chains: Amylose-type starch is a
       basically unbranched polymer consisting of \alpha-1,4-glycosidically
       branched glucose molecules, whereas amylopectin-type starch is
       a mixture of branched glucose chains, comprising additionally
       \alpha-1,6-glycosidic interlinkings.
SUMM
       The molecular structure of starch mainly depends on its degree of
       branching, the amylose/amylopectin ratio, the average
       chain-length, chain length distribution, and degree of phosphorylation,
       further determining the functional properties of the starch and.
SUMM
            . mutant by classical breeding due to the polyploidity of wheat
       (tetra- or hexaploidity). However, a wheat mutant producing waxy-type
       starch (amylose-free starch) was recently achieved
       by breeding methods (Nakamura et al., Mol. Gen. Genet. 248 (1995),
       253-259).
SUMM
            . particular crop plants and most preferred starch-storing
       plants, e.g., rye, barley, oats, wheat, millet, sago, rice, maize, peas,
       wrinkled peas, cassava, potato, tomato, oilseed rape, soy
       bean, hemp, flax, sunflower, cow-pea, arrowroot, clover, ryegrass, or
       alfalfa, in particularly potato, maize, rice.
SUMM
          . . or plant selected from the group consisting of a potato, maize,
       oat, rye, barley, wheat, pea, rice, millet, wrinkled peas,
       cassava, sago, tomato, oilseed rape, soy bean, hemp, flax,
       sunflower, cow-pea, arrowroot, clover, ryegrass, alfalfa, and maniok.
SUMM

    particular a monocotyledonous or dicotyledonous plant cell,

       preferably, a potato, maize, oat, rye, barley, wheat, pea, rice, millet,
       wrinkled peas, cassava, sago, tomato, oilseed rape, soy bean,
       hemp, flax, sunflower, cow-pea, arrowroot, clover, ryegrass, alfalfa, or
       maniok cell, in particular potato,.
SUMM
         . . dicotyledonous plant, preferably a crop plant, in particular a
       rye, barley, oat, rice, wheat, millet, sago, maize, pea, wrinkled pea,
       cassava, potato, tomato, maniok, oil seed rape, soy bean, hemp,
       flax, sunflower, cow-pea, white clover, ryegrass, alfalfa or arrowroot
       plant, most.
SUMM
               ensures transcription and/or translation" may also comprise a
```

```
nucleic acid molecule which leads to a timely and/or locally (endosperm,
       root, tuber, leaf, stem, seed, fruit, apoplast, vacuole,
       cytosol, plastid, mitochondrium, lysosme) limited transcription within a
       plant/or plant cell or which is.
             . e.g., maize, wheat and rice grains or seeds and potato tubers
SUMM
       and the like. For the transformation of potato the tuber
       -specific B33-promoter (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) may
       be used. Apart from promoters, DNA regions initiating transcription may.
SUMM
             . of transcription and/or translation of the nucleic acid
       molecule of the invention in a specific tissue (e.g., endosperm, leaf,
       stem, tuber, meristem, fruit, root, seed) or cell compartiment
       (e.g., cytosol, apoplast, plastid, mitochondrium, vacuole, lysosome).
       Optionally, the term "regulatory element" comprises.
            . the only option. Also solid plastics products, such as pots,
SUMM
       plates and bowls can be produced by means of a starch
       content of more than 50%. Furthermore, the starch/polymer
       mixtures offer the advantage that they are much easier biodegradable.
CLM
       What is claimed is:
          the plant is selected from the group consisting of rye, barley, oat,
       rice, wheat, millet, sago, maize, pea, wrinkled pea, cassava,
       potato, tomato, maniok, oil seed rape, soy bean, hemp, flax, sunflower,
       cow-pea, white clover, ryegrass, alfalfa and arrowroot.
L5
     ANSWER 2 OF 6 USPATFULL
       2001:105536 USPATFULL
ΑN
       Plants which synthesize a modified starch, process for the production
ΤI
       thereof and modified starch
       Kossmann, Jens, Golm, Germany, Federal Republic of
IN ·
       Lorberth, Ruth, Berlin, Germany, Federal Republic of
PΙ
       US 2001007155
                          A1
                                20010705
                                20001221 (9)
AΤ
       US 2000-746390
                          A1
       Division of Ser. No. US 1998-45360, filed on 19 Mar 1998, GRANTED, Pat.
RLI
       No. US 6207880 Continuation of Ser. No. WO 1996-EP4109, filed on 19 Sep
       1996, UNKNOWN
PRAI
       DE 1995-19534759
                           19950919
       DE 1995-19547733
                           19951220
DT
       Utility
FS
       APPLICATION
LN.CNT 1976
       INCLM: 800/284.000
INCL
       INCLS: 536/023.200; 536/102.000; 435/320.100; 435/410.000
NCL
              800/284.000
       NCLS: 536/023.200; 536/102.000; 435/320.100; 435/410.000
IC
       [7]
       ICM: A01H001-00
       ICS: C07H021-04; C12N015-82; C12N015-87; C12N005-00; C12N005-02
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM
       . . . the glucose chains. One differentiates particularly between
       amylose-starch, a basically non-branched polymer made up of
       \alpha-1,4-glycosidically branched glucose molecules, and
       amylopectin-starch which in turn is a mixture of more or less
       heavily branched glucose chains. The branching results from the
       occurrence.
SUMM
       [0004] The molecular structure of starch which is mainly determined by
       its degree of branching, the amylose/amylopectin ratio, the
       average chain-length and the occurrence of phosphate groups is
       significant for important functional properties of starch or,
       respectively,.
SUMM
        . . . and a maize variety (waxy maize) was established by means of
       breeding the starch of which consists of almost 100% amylopectin (Akasuka and Nelson, J. Biol. Chem. 241 (1966), 2280-2285). Furthermore,
```

mutants of potato and pea have been described which synthesize.

SUMM from plants, in particular from starch-synthesizing or starch-storing plants. Cereals (such as barley, rye, oats, wheat etc.), maize, rice, pea, cassava, potato etc. are particularly preferred. They can also be produced by means of synthesis methods known to the skilled person. . . . These are preferably useful plants, in particular SUMM starch-storing plants such as cereals (rye, barley, oats, wheat etc.), rice, maize, peas, cassava and potatoes. heating, a low maximum viscosity as well as almost no increase SUMM in viscosity during cooling (cf. FIG. 7). If the amylose/ amylopectin ratio of this starch is determined, this starch is characterized in that almost no amylose can be measured. The amylose. SUMM . . interest, in particular useful plants and preferably starch-storing plants such as cereals (rye, barley, oats, wheat etc.), rice, maize, pea, cassava and potatoes. SUMM . . potato these parts are the potato seeds, in the case of potatoes the tubers. In order to transform potatoes the tuber -specific B33-promoter (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) may be used particularly, but not exclusively. SUMM the only option. Also solid plastics products, such as pots, plates and bowls can be produced by means of a starch content of more than 50%. Furthermore, the starch/polymer mixtures offer the advantage that they are much easier biodegradable. SUMM . by recombinant DNA techniques are, on the one hand, structure, water content, protein content, lipid content, fiber content, ashes/phosphate content, amylose/amylopectin ratio, distribution of the relative molar mass, degree of branching, granule size and shape as well as crystallization, and on. DETD [0179] 8. Determination of the amylose/amylopectin ratio in starch obtained from potato plants DETD [0180] Starch was isolated from potato plants according to standard methods and the amylose/amylopectin ratio was determined according to the method described by Hovenkamp-Hermelink et al. (Potato Research 31 (1988) 241-246). DETD . starch were dissolved in elution buffer (final volume 80 ml). The starch was derived from potatoes which produce an almost amylose-free starch due to the antisense-expression of a DNA sequence encoding the starch granule-bound starch synthase I (GBSS I) from potato.. DETD [0270] b) Determination of the amylose/amylopectin ratio DETD . . which was isolated from the tubers of transformed potato plants was examined with respect to the ratio of amylose to amylopectin . The plant line R4-1 (shown in line 4 of FIG. 6) exhibited an amylose content of more than 70%. For. [0275] b) Determination of the amylose/amylopectin ratio DETD DETD . which was isolated from the tubers of transformed potato plants was examined with respect to the ratio of amylose to amylopectin

. The plant line R3-5 (shown in line 4 of FIG. 7) exhibited an amylose content of less than 4%. For.

CLMWhat is claimed is:

39. Tuber of a potato plant of claim 38.

40. The tuber of claim 39 which in comparison to tubers of wildtype plants exhibits a reduced cold sweetening.

L5 ANSWER 3 OF 6 USPATFULL

ΑN 2001:44437 USPATFULL

Plants which synthesize a modified starch, process for the production TI thereof and modified starch

IN Kossmann, Jens, Golm, Germany, Federal Republic of Lorberth, Ruth, Berlin, Germany, Federal Republic of

PA Planttec Biotechnologie GmbH, Potsdam, Germany, Federal Republic of

```
(non-U.S. corporation)
PΙ
       US 6207880
                          B1
                               20010327
AΙ
       US 1998-45360
                               19980319 (9)
RLI
       Continuation of Ser. No. WO 1996-EP4109, filed on 19 Sep 1996
                           19950919
PRAI
       DE 1995-19534759
       DE 1995-19547733
                           19951220
DT
       Utility
FS
       Granted
LN.CNT 1933
INCL
       INCLM: 800/284.000
       INCLS: 800/298.000; 435/069.100; 435/101.000; 435/320.100; 435/419.000;
              435/468.000; 435/194.000; 536/023.600
NCL
       NCLM:
              800/284.000
              435/069.100; 435/101.000; 435/194.000; 435/320.100; 435/419.000;
       NCLS:
              435/468.000; 536/023.600; 800/298.000
IC
       [7]
       ICM: C12N015-29
       ICS: C12N015-54; C12N015-82; A01H005-00; C12P019-04
EXF
       536/23.6; 435/101; 435/194; 435/69.1; 435/320.1; 435/419; 435/468;
       800/284; 800/298
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM
         . . the glucose chains. One differentiates particularly between
       amylose-starch, a basically non-branched polymer made up of
       \alpha-1,4-glycosidically branched glucose molecules, and
       amylopectin-starch which in turn is a mixture of more or less
       heavily branched glucose chains. The branching results from the
       occurrence.
SUMM
       The molecular structure of starch which is mainly determined by its
       degree of branching, the amylose/amylopectin ratio, the
       average chain-length and the occurrence of phosphate groups is
       significant for important functional properties of starch or,
       respectively,.
SUMM
             . and a maize variety (waxy maize) was established by means of
       breeding the starch of which consists of almost 100% amylopectin
       (Akasuka and Nelson, J. Biol. Chem. 241 (1966), 2280-2285). Furthermore,
       mutants of potato and pea have been described which synthesize.
SUMM
             . from plants, in particular from starch-synthesizing or
       starch-storing plants. Cereals (such as barley, rye, oats, wheat etc.),
       maize, rice, pea, cassava, potato etc. are particularly
       preferred. They can also be produced by means of synthesis methods known
       to the skilled person.
SUMM
            . These are preferably useful plants, in particular
       starch-storing plants such as cereals (rye, barley, oats, wheat etc.),
       rice, maize, peas, cassava and potatoes.
SUMM
            . heating, a low maximum viscosity as well as almost no increase
       in viscosity during cooling (cf. FIG. 7). If the amylose/
       amylopectin ratio of this starch is determined, this starch is
       characterized in that almost no amylose can be measured. The amylose.
SUMM
            . interest, in particular useful plants and preferably
       starch-storing plants such as cereals (rye, barley, oats, wheat etc.),
       rice, maize, pea, cassava and potatoes.
SUMM
            . potato these parts are the potato seeds, in the case of
       potatoes the tubers. In order to transform potatoes the tuber
       -specific B33-promoter (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) may
       be used particularly, but not exclusively.
SUMM
             . the only option. Also solid plastics products, such as pots,
       plates and bowls can be produced by means of a starch
       content of more than 50%. Furthermore, the starch/polymer
       mixtures offer the advantage that they are much easier biodegradable.
SUMM
            . by recombinant DNA techniques are, on the one hand, structure,
       water content, protein content, lipid content, fiber content,
       ashes/phosphate content, amylose/amylopectin ratio,
       distribution of the relative molar mass, degree of branching, granule
```

```
size and shape as well as crystallization, and on.
DETD
       8. Determination of the Amylose/Amylopectin Ratio in Starch
       Obtained from Potato Plants
DETD
       Starch was isolated from potato plants according to standard methods and
       the amylose/amylopectin ratio was determined according to the
       method described by Hovenkamp-Hermelink et al. (Potato Research 31
       (1988) 241-246).
DETD
                starch were dissolved in elution buffer (final volume 80 ml).
       The starch was derived from potatoes which produce an almost
       amylose-free starch due to the antisense-expression of
       a DNA sequence encoding the starch granule-bound starch synthase I (GBSS
       I) from potato...
DETD
       b) Determination of the Amylose/Amylopectin Ratio
                which was isolated from the tubers of transformed potato plants
DETD
       was examined with respect to the ratio of amylose to amylopectin
       . The plant line R4-1 (shown in line 4 of FIG. 6) exhibited an amylose
       content of more than 70%. For.
DETD
       b) Determination of the Amylose/Amylopectin Ratio
                which was isolated from the tubers of transformed potato plants
DETD
       was examined with respect to the ratio of amylose to amylopectin
       . The plant line R3-5 (shown in line 4 of FIG. 7) exhibited an amylose
       content of less than 4%. For.
CLM
       What is claimed is:
       15. The transgenic plant according to claim 9 which is a rye, barley,
       oat, wheat, rice, maize, pea, cassava or potato plant.
L5
     ANSWER 4 OF 6 USPATFULL
AN
       1999:128830 USPATFULL
TΙ
       Glycogen biosynthetic enzymes in plants
       Stalker, David M., Davis, CA, United States
IN
       Shewmaker, Christine K., Woodland, CA, United States
PΑ
       Calgene, Inc., Davis, CA, United States (U.S. corporation)
_{
m PI}
       US 5969214
                               19991019
ΑI
       US 1995-484434
                               19950607 (8)
RLI
       Continuation of Ser. No. US 1993-16881, filed on 11 Feb 1993 which is a
       continuation-in-part of Ser. No. US 1991-735065, filed on 24 Jul 1991,
       now patented, Pat. No. US 5349123 which is a continuation-in-part of
       Ser. No. US 1990-632383, filed on 21 Dec 1990, now abandoned And Ser.
       No. US 1991-731226, filed on 16 Jul 1991, now abandoned And a
       continuation-in-part of Ser. No. US 1990-536392, filed on 11 Jun 1990,
       now abandoned
DT
       Utility
       Granted
FS
LN.CNT 3046
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       INCLM: 800/205.000
       INCLS: 800/DIG.042; 800/DIG.052; 800/DIG.035; 800/DIG.056; 800/DIG.057;
              536/023.200; 536/023.600; 536/023.700; 536/024.100; 435/069.100;
              435/069.800; 435/101.000; 435/172.300; 435/419.000; 435/412.000;
              435/417.000
NCL
       NCLM:
              800/284.000
       NCLS:
              435/069.100; 435/069.800; 435/101.000; 435/412.000; 435/417.000;
              435/419.000; 536/023.200; 536/023.600; 536/023.700; 536/024.100;
              800/298.000; 800/317.200; 800/320.000; 800/320.100; 800/320.200
IC
       [6]
       ICM: A01H005-00
       ICS: C12N015-31; C12N015-82; C12N015-84; C12P019-04
EXF
       536/23.2; 536/23.6; 536/24.1; 536/23.7; 435/69.1; 435/69.8; 435/172.3;
       435/240.4; 435/97; 435/99; 435/101; 435/194; 435/201-204; 435/419;
       435/412; 435/417; 800/205; 800/DIG.42; 800/52; 800/55-57
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
               form of starch. Starch, a complex polymer of D-glucose,
       consists of a mixture of linear chain (amylose) and branched chain (
       amylopectin) glucans. Starches isolated from different plants
```

are found to have distinct proportions of amylose. Typically, amylose comprises from about 10-25% of plant starch, the remainder being the branched polymer amylopectin. Amylopectin contains low molecular weight chains and high molecular weight chains, with the low molecular weight chains ranging from 5-30 glucose units and the high molecular weight chains from 30-100 or more. The ratio of amylose/ amylopectin and the distribution of low molecular weight to high molecular weight chains in the amylopectin fraction are known to affect the properties, such as thermal stabilization, retrogradation, and viscosity, and therefore the utility of starch. The highest published low m.w./high m.w. chain ratios (on a weight basis) in amylopectin are 3.9/1 for waxy corn starch, which has unique properties. Additionally, duwx, which has slightly more branch points than waxy,. the presence of phosphate groups. In some plant species, mutants have been identified which have altered contents of amylose and amylopectin. Mutations that affect the activity of starch-branching enzyme in peas, for example, result in seeds having less starch and a lower proportion of amylopectin. Also, mutations in the waxy locus of maize, which encodes a starch granule bound starch synthase, result in plants which produce amylopectin exclusively. Similarly, a potato mutant has been identified whose starch is amylose-free (Hovenkamp-Hermelink et al. Theor. Appl. Genet. (1987) 75:217-221). It has been found that varying the degree of starch branching can. It would thus be desirable to develop plant varieties through genetic engineering, which have increased capacity for starch synthesis, altered amylose/amylopectin ratios, altered distribution of low to high molecular weight chains in the amylopectin fraction and also starches with novel molecular weight characteristics. In this manner, useful starches with a variety of viscosity or. ( act. ( act. ) host plant for such transformation is potato because of the large amount of starch production in potato tubers. A typical tuber contains approximately 16% of its fresh weight as starch (Burton, W. G., The Potato (1966) 3rd Edition, Longman Scientific and Technical Publications, England, p. 361). Transformation of potato plants with the bacterial CGT structural gene linked to a tuber -specific promoter and a leader directing the enzyme, for example, to the amyloplast, provides a means to produce large quantities of. . it can be recognized that the modulation of polysaccharide modification enzymes in these plant cells has implications for modifying the starch content and/or composition of these cells. In this manner, plants or plant parts which synthesize and store starch may be obtained which have increased or decreased starch content and modified starch related properties such as specific gravity, free sugar content and/or novel and useful starches. In particular, potato starch having decreased amylose and modified amylopectin may be produced and further applications to modify starches consisting entirely of amylopectin such as that of waxy maize or a mutant potato, are also considered. Similarly, the starch from these plant parts. . . is modified. In particular, the percentage of amylose is decreased and the ratio of low m.w./high m.w. chains in the amylopectin fraction is increased. This phenotypic effect in planta is indicative of glgA biological activity. Additional disclosure

SUMM

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SUMM

concerning glycogen biosynthetic enzymes. . . . (Chemistry and Industry, London (1988) 13:410). In addition, it has been discovered that in vitro application of  $\beta\text{-CDs}$  to potato tuber slices inhibits discoloration, and in vitro application to whole potato tubers prevents a typical blackspot reaction caused by bruising. Additional. . .

SUMM . . . of the protein (e.g. Western or ELISA), as a result of phenotypic changes observed in the cell, such as altered **starch content**, altered starch branching, etc., or by assay for

increased enzyme activity, and the like. If desired the enzyme may be. SUMM the invention is a transcriptional initiation region from the patatin gene of potato, which demonstrates preferential expression in the potato tuber. Similarly, other promoters which are preferentially expressed in the starch-containing tissues, such as the zein genes in corn, as opposed. SUMM . . . in the root structures, such as potato (e.g., Irish (Solanum tuberosum), Sweet (Ipomoea batatas), and yam (Discorea spp.)), tapioca (e.g. cassava (Manihot esculenta)) and arrowroot (e.g., Marantaceae spp., Cycadaceae spp., Cannaceae spp., Zingiberaceae spp., etc.), or in the stem, such as. . SUMM in the plant cells or parts. These alterations may include modification of dry matter content, free sugar content or of starch content and/or structure, or modification of specific gravity. The novel plant cells or plant parts can thus be harvested and used. SUMM localized in the starch storage organelles, tissues or regions of the host plant, e.g., the amyloplast of a host potato tuber . The structural gene will manifest its activity by mediating the production of degradation products in at least one portion of. . in sterile distilled water and kept immersed in sterile liquid DETD MS medium (#1118, Gibco; Grand Island; N.Y.) to prevent browning. Tuber discs (1-2 mm thick) are prepared by cutting columns of potato tuber with a .about.1 cm in diameter cork borer and slicing the columns into discs of the desired thickness. Discs are. DETD . transformation and regeneration culture and growth conditions described above in production of glgA transformed plants. To compare values from each tuber sample, the specific gravity measurements are converted to reflect % total solids content of tubers. Percent total solids is calculated. DETD analysis may be conducted on tubers from selected pCGN1457 and pCGN1457B transformed plants and from non-transformed controls (RB-43) to determine starch content, amylose percentages and to elucidate chain length distribution in the amylopectin component of the starch. Starch granules are isolated as described by Boyer et al. (1976) Cereal Chemistry 53:327-337) and starch content estimated on a weight basis (starch wt/fresh wt).

Analyses of Trangenic Potato Tuber Starch

al. (1985) Starch/Starke.

% Low

Amylose percentages are determined by gel-filtration analysis (Boyer et

High Low M.W./

Spec. % % M.W. M.W. High

Construct Gravity Starch Amylose Chains Chains M.W.

TABLE 2

RB-43.

DETD

DETD . . . also have altered starch. In particular, the percentage of amylose in the transgenic potato tubers is decreased. In addition, the amylopectin portion of the starch from transgenic potato tubers has more low molecular weight chains and less high molecular weight chains than wild type potato tuber amylopectin, thus indicating that the amylopectin from transgenic tubers has more branch points.

DETD . . . glycogen biosynthetic enzymes in plants has been shown to result in starch having altered properties, in particular altered ratios of amylose/amylopectin and altered distribution of low molecular weight chain lengths to high molecular weight chain lengths in the amylopectin fraction. In this manner, plants, including plant cells and plant parts, having modified starch properties may be obtained, wherein the. . .

```
DETD
       Tuber discs (1-2 mm thick) are prepared by cutting columns of
       potato tuber with a 1 cm cork borer and slicing the columns to
       the desired thickness. Discs are placed into the liquid.
       Total RNA is isolated from 5 g of tuber tissue (as described
DETD
       by Logeman et al., Anal. Biochem. (1987) 163:16-20). Poly-(A)+RNA is
       purified over oligo(dT) cellulose (as described by.
DETD
       To prepare samples for chromatography, cubes of frozen tuber
       tissue are ground into a powder in a coffee mill (Krups®, Closter,
       N.J.). For each plant assayed, extracts from tubers.
DETD
                are 0.39 for \alpha-CD and 0.36 for \beta-CD. The \alpha-CD
       band stained light violet, while the \beta-CD band stained yellow.
       Tuber tissue from 20 transformed plants is screened for the
       presence of \alpha-CD and \beta-CD. Tissue of tubers from eight
       Russet.
CLM
       What is claimed is:
          claim 4, wherein said transcription and translational initiation
       regions are from a gene which is preferentially expressed in a potato
       tuber.
       17. The method of claim 16 wherein said modified starch has an altered
       amylose to amylopectin ratio as compared to that of a control
       starch storage organ.
          of claim 16 wherein said modified starch has an altered ratio of low
       to high molecular weight chains in the amylopectin fraction as
       compared to that of a control starch storage organ.
     ANSWER 5 OF 6 USPATFULL
L5
AN
       1998:51944 USPATFULL
ΤI
       Glycogen biosynthetic enzymes in plants
IN
       Stalker, David M., 2736 Cumberland Pl., Davis, CA, United States
       Shewmaker, Christine K., 1409 Spring Creek, Woodland, CA, United States
       Oakes, Janette V., 2408 Amapola Dr., Davis, CA, United States 95616
PΙ
       US 5750875
                               19980512
ΑI
       US 1995-469202
                               19950606 (8)
RLI
       Continuation-in-part of Ser. No. US 1990-536392, filed on 11 Jun 1990,
       now abandoned And a continuation of Ser. No. US 1993-16881, filed on 11
       Feb 1993 which is a continuation-in-part of Ser. No. US 1991-735065,
       filed on 24 Jul 1991, now patented, Pat. No. US 5349123 which is a
       continuation-in-part of Ser. No. US 1990-632383, filed on 21 Dec 1990,
       now abandoned And Ser. No. US 1991-731226, filed on 16 Jul 1991, now
       abandoned
DT
       Utility
FS
       Granted
LN.CNT 2684
INCL
       INCLM: 800/205.000
       INCLS: 800/DIG.042; 800/DIG.052; 800/DIG.055; 800/DIG.056; 800/DIG.057;
              435/069.100; 435/069.800; 435/097.000; 435/101.000; 435/172.300;
              435/412.000; 435/417.000; 435/419.000; 536/023.200; 536/023.600;
              536/023.700; 536/024.100
NCL
              800/284.000
       NCLM:
       NCLS:
              435/069.100; 435/069.800; 435/097.000; 435/101.000; 435/412.000;
              435/417.000; 435/419.000; 536/023.200; 536/023.600; 536/023.700;
              536/024.100; 800/288.000; 800/298.000
IC
       [6]
       ICM: A01H005-00
       ICS: C12N015-82; C12N015-84; C12N015-31; C12P019-04
EXF
       536/23.2; 536/23.6; 536/24.1; 536/23.7; 435/69.1; 435/69.8; 435/172.3;
       435/240.4; 435/97; 435/99; 435/101; 435/201-204; 435/419; 435/412;
       435/417; 800/205; 800/DIG.42; 800/52; 800/55; 800/56; 800/57
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
         . . form of starch. Starch, a complex polymer of D-qlucose,
```

consists of a mixture of linear chain (amylose) and branched chain ( amylopectin) glucans. Starches isolated from different plants are found to have distinct proportions of amylose. Typically, amylose comprises from about 10-25% of plant starch, the remainder being the branched polymer amylopectin. Amylopectin contains low molecular weight chains and high molecular weight chains, with the low molecular weight chains ranging from 5-30 glucose units and the high molecular weight chains from 30-100 or more. The ratio of amylose/ amylopectin and the distribution of low molecular weight to high molecular weight chains in the amylopectin fraction are known to affect the properties, such as thermal stabilization, retrogradation, and viscosity, and therefore the utility of starch. The highest published low m.w./high m.w. chain ratios (on a weight basis) in amylopectin are 3.9/1 for waxy corn starch, which has unique properties. Additionally, duwx, which has slightly more branch points than waxy,. the presence of phosphate groups. In some plant species, mutants have been identified which have altered contents of amylose and amylopectin. Mutations that affect the activity of starch-branching enzyme in peas, for example, result in seeds having less starch and a lower proportion of amylopectin. Also, mutations in the waxy locus of maize, which encodes a starch granule bound starch synthase, result in plants which produce amylopectin exclusively. Similarly, a potato mutant has been identified whose. starch is amylose-free (Hovenkamp-Hermelink et al. Theor. Appl. Genet. (1987) 75:217-221). It has been found that varying the degree of starch branching can. It would thus be desirable to develop plant varieties through genetic engineering, which have increased capacity for starch synthesis, altered amylose/amylopectin ratios, altered distribution of low to high molecular weight chains in the amylopectin fraction and also starches with novel molecular weight characteristics. In this manner, useful starches with a variety of viscosity or. host plant for such transformation is potato because of the large amount of starch production in potato tubers. A typical tuber contains approximately 16% of its fresh weight as starch (Burton, W. G., The Potato (1966) 3rd Edition, Longman Scientific and Technical Publications, England, p. 361). Transformation of potato plants with the bacterial CGT structural gene linked to a tuber -specific promoter and a leader directing the enzyme, for example, to the amyloplast, provides a means to produce large quantities of. . it can be recognized that the modulation of polysaccharide modification enzymes in these plant cells has implications for modifying the starch content and/or composition of these cells. In this manner, plants or plant parts which synthesize and store starch may be obtained which have increased or decreased starch content and modified starch related properties such as specific gravity, free sugar content and/or novel and useful starches. In particular, potato starch having decreased amylose and modified amylopectin may be produced and further applications to modify starches consisting entirely of amylopectin such as that of waxy maize or a mutant potato, are also considered. Similarly, the starch from these plant parts. . . is modified. In particular, the percentage of amylose is decreased and the ratio of low m.w./high m.w. chains in the amylopectin fraction is increased. This phenotypic effect in planta is indicative of glgA biological activity. Additional disclosure concerning glycogen biosynthetic enzymes. (Chemistry and Industry, London (1988) 13:410). In addition, it has been discovered that in vitro application of  $\beta$ -CDs to potato tuber slices inhibits discoloration, and in vitro application to whole potato tubers prevents a typical blackspot reaction caused by

. . of the protein (e.g. Western or ELISA), as a result of

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SUMM

DETD

DETD

DETD

bruising. Additional.

phenotypic changes observed in the cell, such as altered starch content, altered starch branching, etc., or by assay for increased enzyme activity, and the like. If desired the enzyme may be.
. . . the invention is a transcriptional initiation region from the

DETD . . . the invention is a transcriptional initiation region from the patatin gene of potato, which demonstrates preferential expression in the potato tuber. Similarly, other promoters which are preferentially expressed in the starch-containing tissues, such as the zein genes in corn, as opposed. . .

DETD . . . in the root structures, such as potato (e.g., Irish (Solanum tuberosum), Sweet (Ipomoea batatas), and yam (Discorea spp.)), tapioca (e.g. cassava (Manihot esculenta)) and arrowroot (e.g., Marantaceae spp., Cycadaceae spp., Cannaceae spp., Zingiberaceae spp., etc.), or in the stem, such as. . .

DETD . . . in the plant cells or parts. These alterations may include modification of dry matter content, free sugar content or of starch content and/or structure, or modification of specific gravity. The novel plant cells or plant parts can thus be harvested and used. . .

DETD . . . localized in the starch storage organelles, tissues or regions of the host plant, e.g., the amyloplast of a host potato **tuber** . The structural gene will manifest its activity by mediating the production of degradation products in at least one portion of. . .

DETD . . . in sterile distilled water and kept immersed in sterile liquid MS medium (#1118, Gibco; Grand Island; N.Y.) to prevent browning.

Tuber discs (1-2 mm thick) are prepared by cutting columns of potato tuber with a .about.1 cm in diameter cork borer and slicing the columns into discs of the desired thickness. Discs are. .

DETD . . . transformation and regeneration culture and growth conditions described above in production of glgA transformed plants. To compare values from each tuber sample, the specific gravity measurements are converted to reflect % total solids content of tubers. Percent total solids is calculated. . .

DETD . . . analysis may be conducted on tubers from selected pCGN1457 and pCGN1457B transformed plants and from non-transformed controls (RB-43) to determine starch content, amylose percentages and to elucidate chain length distribution in the amylopectin component of the starch. Starch granules are isolated as described by Boyer et al. (1976) Cereal Chemistry 53:327-337) and starch content estimated on a weight basis (starch wt/fresh wt).

Amylose percentages are determined by gel-filtration analysis (Boyer et al. (1985) Starch/Starke. . .

DETD TABLE 2

Analyses of Trangenic Potato Tuber Starch

% % Low
High Low M.W./

Spec. % % M.W. M.W. High

Construct

Gravity Starch Amylose

Chains

Chains

M.W.

RB-43 1.081.

DETD . . . also have altered starch. In particular, the percentage of amylose in the transgenic potato tubers is decreased. In addition, the amylopectin portion of the starch from transgenic potato tubers has more low molecular weight chains and less high molecular weight chains than wild type potato tuber amylopectin, thus indicating that the amylopectin from transgenic tubers has more branch points.

DETD . . . glycogen biosynthetic enzymes in plants has been shown to

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of amylose/amylopectin and altered distribution of low
       molecular weight chain lengths to high molecular weight chain lengths in
       the amylopectin fraction. In this manner, plants, including
      plant cells and plant parts, having modified starch properties may be
       obtained, wherein the.
DETD
       Tuber discs (1-2mm thick) are prepared by cutting columns of
      potato tuber with a 1 cm cork borer and slicing the columns to
       the desired thickness. Discs are placed into the liquid.
       Total RNA is isolated from 5 g of tuber tissue (as described
DETD
       by Logeman et al., Anal. Biochem. (1987) 163:16-20). Poly-(A)+RNA is
      purified over oligo(dT) cellulose (as described by.
DETD
            . nitrogen, and stored at approximately -70° C until
       assayed. Extraction of Cyclodextrin To prepare samples for
       chromatography, cubes of frozen tuber tissue are ground into a
       powder in a coffee mill (KrupsO, Closter, N.J.). For each plant assayed,
       extracts from tubers.
DETD
               are 0.39 for \alpha-CD and 0.36 for \beta-CD. The \alpha-CD
       band stained light violet, while the β-CD band stained yellow.
       Tuber tissue from 20 transformed plants is screened for the
       presence of \alpha\text{-CD} and \beta\text{-CD}. Tissue of tubers from eight
       Russet.
CLM
       What is claimed is:
         claim 4, wherein said transcription and translational initiation
       regions are from a gene which is preferentially expressed in a potato
       tuber.
L5
     ANSWER 6 OF 6 USPATFULL
AN
       94:82416 USPATFULL
TI
       Glycogen biosynthetic enzymes in plants
IN
       Shewmaker, Christine K., Woodland, CA, United States
       Stalker, David M., Davis, CA, United States
       Calgene, Inc., Davis, CA, United States (U.S. corporation)
PA
       US 5349123
PΙ
                               19940920
       US 1991-735065
ΑI
                               19910724 (7)
RLT
       Continuation-in-part of Ser. No. US 1990-632383, filed on 21 Dec 1990,
       now abandoned And a continuation-in-part of Ser. No. US 1991-731226,
       filed on 16 Jul 1991, now abandoned
DT
       Utility
FS
       Granted
LN.CNT 1361
       INCLM: 800/205.000
INCL
       INCLS: 800/DIG.041; 800/DIG.052; 800/DIG.055; 800/DIG.056; 800/DIG.057;
              435/069.100; 435/069.800; 435/070.100; 435/172.300; 435/240.400;
              536/023.200; 536/023.700; 536/024.100; 536/023.400
NCL
              800/284.000
       NCLM:
       NCLS:
              435/069.100; 435/069.800; 435/070.100; 435/412.000; 435/417.000;
              435/419.000; 536/023.200; 536/023.400; 536/023.700; 536/024.100;
              800/298.000; 800/317.200; 800/320.000; 800/320.100; 800/320.200
IC
       [5]
       ICM: A01H001-04
       ICS: C12N015-00; C07H021-04; C12P021-04
EXF
       435/69.1; 435/69.8; 435/70.1; 435/172.3; 435/240.4; 435/320.1; 435/183;
       536/27; 536/23.2; 536/23.7; 536/24.1; 536/23.4; 800/205; 800/DIG.42;
       800/DIG.52; 800/DIG.55; 800/DIG.56; 800/DIG.57
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM
             . a complex polymer of glucose, consists of a mixture of linear
       chain and branched chain glucans known as amylose and
       amylopectin respectively. Starches isolated from different
       plants are found to have variable contents of amylose. Typically,
       amylose comprises from about 10-25% of plant starch, the remainder being
```

the branched polymer amylopectin. Amylopectin

contains low molecular weight chains and high molecular weight chains,

result in starch having altered properties, in particular altered ratios

with the low molecular weight chains ranging from 5-30 glucose units and the high molecular weight chains from 30-100 or more. The ratio of amylose/amylopectin and the distribution of low molecular weight to high molecular weight chains in the amylopectin fraction are known to affect the properties, such as thermal stabilization, retrogradation, and viscosity, and therefore utility of starch. The highest published low m.w./high m.w. chain ratios (on a weight basis) in amylopectin are 3.9/1 for waxy corn starch which has unique properties. Additionally, duwx, which has slightly more branch points than waxy. the presence of phosphate groups. In some plant species, mutants have been identified which have altered contents of amylose and amylopectin. Mutations that affect the activity of starch-branching enzyme in peas, for example, result in seeds having less starch and a lower proportion of amylopectin. Also, mutations in the waxy locus of maize, which encodes a starch granule bound starch synthase, result in plants which produce amylopectin exclusively. Similarly, a potato mutant has been identified whose starch is amylose-free (Hovenkamp-Hermelink et al. Theor. Appl. Genet. (1987) 75:217-221). It has been found that varying the degree of starch branching can. It would thus be desirable to develop plant varieties through genetic engineering, which have increased capacity for starch synthesis, altered amylose/amylopectin ratios, altered distribution of low to high molecular weight chains in the amylopectin fraction and also starches with novel molecular weight characteristics. In this manner, useful starches with a variety of viscosity or. it can be recognized that the modulation of glycogen biosynthetic enzymes in these plant cells has implications for modifying the starch content and/or composition of these cells. In this manner, plants or plant parts which synthesize and store starch may be obtained which have increased or decreased starch content and modified starch related properties such as specific gravity, free sugar content and/or novel and useful starches. In particular, potato starch having decreased amylose and modified amylopectin may be produced and further applications to modify starches consisting entirely of amylopectin such as that of waxy maize or a mutant potato, are also considered. Similarly, the starch from these plant parts. . . is modified. In particular, the percentage of amylose is decreased and the ratio of low m.w./high m.w. chains in the amylopectin fraction is increased. This phenotypic effect in planta is indicative of glgA biological activity. . of the protein (e.g. Western or ELISA), as a result of phenotypic changes observed in the cell, such as altered starch content, altered starch branching, etc., or by assay for increased enzyme activity, and the like. If desired the enzyme may be. . the invention is a transcriptional initiation region from the patatin gene of potato, which demonstrates preferential expression in the potato tuber. Similarly, other promoters which are preferentially expressed in the starch-containing tissues, such as the zein genes in corn, as opposed. . and store reserve starch. Plants of interest include, but are not limited to corn, cereal grains, sorghum, rice, potato, tapioca, cassava, arrowroot and sago. . in the plant cells or parts. These alterations may include modification of dry matter content, free sugar content or of starch content and/or structure, or modification of specific gravity. The novel plant cells or plant parts can thus be

**Tuber** discs (1-2 mm thick) are prepared by cutting columns of potato **tuber** with a .about.1 cm in diameter cork borer and

slicing the columns into discs of the desired thickness. Discs are.

SUMM

SUMM

DETD

DETD

DETD

DETD

DETD

DETD

DETD

harvested and used.

DETD . . . transformation and regeneration culture and growth conditions described above in production of glgA transformed plants. To compare values from each tuber sample, the specific gravity measurements are converted to reflect % total solids content of tubers. Percent total solids is calculated. . .

DETD . . . analysis may be conducted on tubers from selected pCGN1457 and pCGN1457B transformed plants and from non-transformed controls (RB-43) to determine starch content, amylose percentages and to elucidate chain length distribution in the amylopectin component of the starch. Starch granules are isolated as described by Boyer et al. (1976) Cereal Chemistry 53:327-337) and starch content estimated on a weight basis (starch wt/fresh wt). Amylose percentages are determined by gel-filtration analysis (Boyer et al. (1985) Starch/Starke. . .

DETD TABLE 2

Analyses of Trangenic Potato Tuber Starch

% % Low
Spec. High Low M.W./
Gravi- % % M.W. M.W. High

Construct

ty Starch Amylose

Chains

Chains

M.W.

RB-43.

DETD . . . also have altered starch. In particular, the percentage of amylose in the transgenic potato tubers is decreased. In addition, the amylopectin portion of the starch from transgenic potato tubers has more low molecular weight chains and less high molecular weight chains than wild type potato tuber amylopectin, thus indicating that the amylopectin from transgenic tubers has more branch points.

DETD . . . glycogen biosynthetic enzymes in plants has been shown to result in starch having altered properties, in particular altered ratios of amylose/amylopectin and altered distribution of low molecular weight chain lengths to high molecular weight chain lengths in the amylopectin fraction. In this manner, plants, including plant cells and plant parts, having modified starch properties may be obtained, wherein the. . .

CLM What is claimed is:

- . plant cell of claim 6 wherein said plant is selected from the group consisting of corn, sorghum, rice, potato, tapioca, cassava, arrowroot, and sago.
- 11. A method to modify a potato **tuber**, wherein said method comprises growing a potato plant, under conditions that will permit the formation of a potato **tuber**, wherein the genome of said potato plant comprises a construct according to claim 3, and wherein said transcription initiation region. . .
- . claim 11 wherein expression of said E. coli glycogen synthase results in modification of the starch composition of said potato  ${\it tuber}$
- 13. The method of claim 12 wherein said modified starch has an altered amylose to **amylopectin** ratio as compared to that of a control starch storage organ.
- 14. The method of claim 11 wherein said potato **tuber** comprises modified starch having an altered ratio of low to high molecular weight chains in the **amylopectin** fraction of said starch, as compared to that of a control potato **tuber**.

- 15. A potato tuber having modified starch, wherein said starch modification consists of an increased percentage of low molecular weight chains in the amylopectin portion of said starch, and wherein said potato tuber is produced according to the method of claim 11.
- 16. The potato tuber of claim 15, wherein the amylopectin fraction of the starch in said potato tuber has a ratio of low to high molecular weight chains of at least 4.0.